Decoupling the effects of hyperbaric pressure and hyperoxygenation on human keratinocyte behaviour

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Introduction

Hyperbaric oxygen (HBO) therapy (i.e. the inspiration of pure oxygen typically at 2–3 ATA) is used to treat certain types of chronic wounds [1]. The promotion of wound healing by HBO is largely attributed to increased oxygen solubility in the plasma under heightened pressure according to Henry's Law, leading to enhanced tissue oxygenation. There has been little literature on the potential cellular effects of hyperbaric pressure alone. Here, we have decoupled hyperbaric pressurisation and hyperoxygenation to ascertain if they might exert independent effects on the growth behaviour of human keratinocytes in wound re-epithelialisation.

Materials and methods

Human keratinocytes (HaCaT) were cultured under normal atmospheric pressure (1 ATA) and 5% CO_2/95% air. HBO, hyperbaric air (HBA) or normobaric oxygen (NBO) were administered once daily for 2 h over up to 48 h (Table 1). Oxygen concentration in the culture medium was determined using a Jenway 970 dissolved oxygen meter. Cell migration and proliferation were assessed using the scratch assay and tetrazolium (MTT) assay, respectively. Cellular expressions of E-cadherin (cell adhesion marker), proliferating cell nuclear antigen (PCNA, cell proliferation marker) and involucrin (cell differentiation

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Table 1: HaCaT cell treatments.

Treatment	O_2 (%)	CO_2 (%)	N_2 (%)	Pressure (ATA)
NBO	95	5	0	1
HBO	95	5	0	3
HBA	21	5	74	3

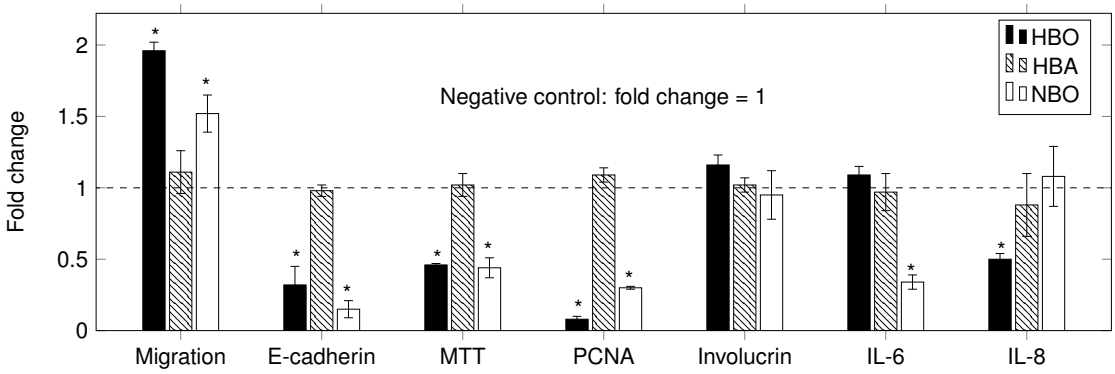

Figure 1: Changes in markers of HaCaT cell behaviour ($n = 3$; $^*p < 0.05$ versus untreated control, which is assigned fold change = 1).

marker) were determined by Western blotting. Interleukin (IL)-6 and IL-8 release into the cell culture supernatant was quantified using enzyme-linked immunosorbent assays (ELISA).

Results and discussion

Maximum dissolved oxygen concentrations were 9.1 mg/mL (HBO), 6.5 mg/mL (NBO) and 1.9 mg/mL (HBA). HBO and NBO were associated with enhanced cell migration and reduced cell proliferation, with simultaneous downregulation of E-cadherin and PCNA (Figure 1). Cell differentiation was unaltered by any treatment. IL-6 release was reduced only with NBO, whilst IL-8 release was reduced only with HBO. Thus, the effects of HBO and NBO on HaCaT cell migration and proliferation are attributable to hyperoxygenation and not hyperbaric pressurisation. However, hyperbaric pressurisation may synergise with hyperoxygenation to exert an immunomodulatory effect on the cells.

Conclusion

HBO and NBO exert similar effects on keratinocyte migration and proliferation. Further research should establish whether NBO can be a viable alternative treatment, to potentially avoid pressure-related adverse effects associated with HBO, such as barotrauma.

References

[1] P. Kranke, M.H. Bennett, M. Martyn-St James, A. Schnabel, S.E. Debus and S. Weibel, “Hyperbaric oxygen therapy for chronic wounds” Cochrane Database Syst. Rev. (2015) CD004123.